Kidney International, Vol. 19 (1981), pp. 678–686

Effect of aldosterone on the transepithelial potential difference of the rat distal tubule

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Effect of aldosterone on the transepithelial potential difference of the rat distal tubule. Aldosterone, which increases sodium reabsorption along the distal nephron of the rat kidney, has not previously been shown to influence the transepithelial potential difference (PD) of this nephron segment in vivo. In contrast, major changes in transepithelial PD have been shown to accompany increases in sodium transport induced by aldosterone in other mineralocorticoid-sensitive epithelia such as the frog skin, toad bladder, and mammalian colon. The present micropuncture study reinvestigates the effect of aldosterone on the PD of both early and late segments of the distal tubule of the rat kidney. Electrodes with tips of 3 to 5 μ in external diameter were used for PD measurements. The mean free-flow PD in early distal segments was +5.9 ± 1.1 mV in intact animals and +11.3 ± 1.1 mV in adrenalectomized animals, whereas in late distal segments it was −19.2 ± 1.9 mV in intact animals and −2.5 ± 1.0 mV in the adrenalectomized groups. Infusion of aldosterone to intact animals produced no significant change in the early distal segments but increased the free-flow PD in late distal segments from −18.3 ± 1.9 to −29.7 ± 2.0 mV ($P<0.001$). In adrenalectomized animals, aldosterone decreased the early segment PD from +11.3 ± 1.1 to +7.4 ± 1.0 mV ($P<0.001$) and increased the late segment PD from −2.5 ± 1.0 to −10.3 ± 1.2 mV ($P<0.001$). Additional distal microperfusion studies designed to eliminate the influence of variations in distal ion delivery on the PD were performed in both intact and adrenalectomized animals. In these studies, PD responses to aldosterone in both early and late distal segments closely resembled those noted under free-flow conditions. These data confirm the recently reported functional heterogeneity of the distal tubule of the rat kidney and demonstrate that in both early and late segments of the distal tubule aldosterone does have a highly significant effect on the transepithelial PD that is consistent with its effect on sodium reabsorption along these segments.

Effet de l'aldostérone sur la différence de potentiel transépithéliale du tube distal du rat. Il n'a pas été montré in vivo que l'aldostérone, qui augmente la réabsorption de sodium le long du tube distal du néphron de rat, influence la différence de potentiel (PD) de ce segment. Au contraire, des modifications importantes de la PD transépithéliale ont été constatées en même temps que l'augmentation du transport de sodium dans d'autres épithéliums sensibles aux minéralo-corticoïdes tels que la peau de grenouille, la vessie de crapaud, et le colon des mammifères. Cette étude par microponctions reprend l'évaluation des effets de l'aldostérone sur la PD des segments précoces et tardifs du tube distal du rein de rat. Des électrodes avec des diamètres de pointe de 3 à 5 μ ont été utilisées pour les mesures de PD. La PD moyenne en flux libre dans le segment distal précoce était de 5,9 ± 1,1 mV chez les animaux intacts et de 11,3 ± 1,1 mV chez les animaux surrénalectomisés alors que dans les segments distaux tardifs la PD moyenne était de − 19,2 ± 1,9 mV chez les animaux intacts et de − 2,5 ± 1,0 mV chez les animaux surrénalectomisés. La perfusion d'aldostérone aux animaux intacts n'a pas produit de modification significative dans les segments distaux précoces mais a augmenté la PD des segments tardifs de − 18,3 ± 1,9 à − 29,7 ± 2,0 mV ($P<0,001$). Chez les animaux surrénalectomisés l'aldostérone diminue la PD des segments précoces de 11,3 ± 1,1 à 7,4 ± 1,0 mV ($P < 0,001$) alors qu'elle augmente celle des segments tardifs de − 2,5 ± 1,0 à − 10,3 ± 1,2 mV ($P < 0,001$). Des études par microperfusion ayant pour but d'éliminer l'influence des variations dans le débit distal d'ions sur la PD ont été réalisées à la fois chez des animaux intacts et des animaux surrénalectomisés. Dans ces travaux la réponse de la PD à l'aldostérone aussi bien dans les segments précoces que tardifs est trés proche de celle obtenue dans des conditions de free flow. Ces résultats confirment les observations récentes sur l'hétérogénéité du tube distal du rat et démontrent qu'aussi bien dans les segments précoces que tardifs du tube distal l'aldostérone a réellement un effet trés significatif sur la PD transépithéliale qui est en accord avec son effet sur la réabsorption du sodium le long de ces segments.

Received for publication May 14, 1979
and in revised form August 20, 1980

0085-2538/81/0019-0678 $01.80

The mineralocorticoid hormone aldosterone is important in the overall regulation of sodium, potassium, and hydrogen ion balance. Excess renal sodium losses and potassium retention accompany mineralocorticoid deficiency, and these abnormalities can be corrected readily by administration of aldosterone or one of its analogues [1–8].

Renal micropuncture studies in rats have clearly demonstrated that the major, if not the sole, site of action of aldosterone in the kidney is the distal nephron [4, 9–12]. Such studies have shown that in the distal tubule of adrenalectomized animals, sodium reabsorption and potassium secretion are both decreased compared with control animals [4, 13]. Furthermore, these defects in distal sodium reabsorption and potassium secretion in the adrenalectomized group are corrected readily by administration of aldosterone [4, 13].

The specific effect of aldosterone on ion transport has been studied extensively using such isolated epithelia as frog skin [14, 15] and toad bladder [16–18], which are frequently regarded as amphibian counterparts of the mammalian distal nephron [19]. Application of aldosterone to the epithelial side of the frog skin or mucosal side of the toad bladder leads to enhanced transmembrane sodium transport [16–18], reflected by an increase in both the transepithelial potential difference (PD) and short-circuit current [16–18]. In view of these findings, it might be expected that mineralocorticoid-dependent changes in sodium reabsorption in the distal nephron of the mammalian kidney would also be accompanied by changes in the transepithelial PD. In previous in vivo micropuncture studies in the rat, however, no such change in PD has been demonstrated in superficial distal tubules under free-flow conditions. In a study reported in 1965, Hierholzer et al [4] found a mean distal PD of −45.2 mV in control animals and a similar PD of −47.4 mV in adrenalectomized animals despite a marked decrease in distal tubular sodium reabsorption in the adrenalectomized group.

This apparent lack of effect of mineralocorticoids on distal tubular PD is surprising but might possibly relate to unsatisfactory electrode techniques [20–23]. In recent years, the electrophysiology of the nephron has been reexamined using electrodes with relatively large tips of 3 to 5 μ in external diameter in contrast to the traditional Ling Gerard electrode. Using such electrodes, Barratt et al [24] reexamined the electrophysiology of the distal tubule of the rat kidney and reported a significantly positive PD of approximately +4 mV in early distal segments and a significantly negative PD of approximately −20 mV in late segments. Other workers have used similar electrodes to investigate the proximal tubule [20, 25], ascending limb of Henle's loop [26, 27], and medullary collecting duct [28, 29].

Although some controversy still exists over the use of large tip electrodes [30–32], we have recently provided data to validate their use in distal PD measurements [33]. On this basis, the present study was designed to reinvestigate the influence of mineralocorticoids on the transepithelial PD of distal segments of surface nephrons in the rat kidney. Highly significant changes in transepithelial PD were observed in early and late distal segments under both free-flow and microperfusion conditions, consistent with an effect of mineralocorticoids in enhancing sodium reabsorption in these segments.

Methods

Studies were performed on 29 male members (body wt, 230 to 335 g) of a locally inbred strain of Ginger hooded rats anesthetized i.p. with Inactin (Promonta, Germany) and prepared for micropuncture of the left kidney as described previously [20]. Ringer's bicarbonate solution (per liter: 140 mmoles of sodium, 5 mmoles potassium, 115 mmoles chloride, and 30 mmoles of bicarbonate) was infused at 0.1 ml/min throughout each experiment.

All animals were fed regular rat chow. Animals of groups 3 and 5 (see below) were bilaterally adrenalectomized with a lumbar approach at least 10 days prior to micropuncture. Following adrenalectomy, these animals received 0.9% saline drinking solution and a weekly i.m. injection of dexamethasone (20 μg/100g of body wt). A similar dose of dexamethasone was administered to all animals of groups 2 through 5 on the day of micropuncture.

Arterial blood samples were collected at the beginning of each experiment and analyzed for sodium and potassium concentration with a Beckman flame photometer.

Five groups of animals were studied. In groups 1 to 3, only free-flow measurements were made, whereas in groups 4 and 5, additional distal microperfusion data were obtained.

Free-flow studies

Group 1: Distal nephron morphology group (5 rats). This group was studied to ascertain that distal segments of surface nephrons in the Ginger hooded strain of rats used for the present experiments were functionally and morphologically similar to those segments previously studied in mutant Wistar rats [24]. Specifically, following a PD measurement in a distal segment, the segment was identified as either early or late distal tubule by perfusing a proximal tubular segment of the same nephron (usually immediately adjacent to the distal segment) with Ringer's bicarbonate solution colored with 0.2% FD & C dye. At the end of each experiment, relevant tubules were perfuse-fixed with a gluteraldehyde-formaldehyde solution [24, 34, 35], and sites of PD measurements were marked by microinjection of latex. Tubules were subsequently microdissected and processed for light and electron microscopic examination. Puncture sites were located in serial tissue sections examined by light microscopy, and finally the tubular morphology at the puncture site was identified by electron microscopy. These techniques have been described in detail previously [24, 34, 35].

Group 2: Aldosterone-infused intact animals (5 rats). Animals of this group were maintained on regular rat chow and tap water. Distal PD measurements were made during an initial control period and subsequently during an experimental period during which aldosterone was administered i.v. in a dose of 1 μg/kg of body wt per hour following an initial bolus of 1 μg/kg of body wt. Measurements were commenced at least 2 hours after the bolus of aldosterone.

Group 3: Aldosterone-infused adrenalectomized animals (7 rats). This group received 0.9% sodium chloride drinking solution. Distal PD measurements were made during an initial control period and subsequently during an experimental period during which aldosterone was administered i.v. in a dose of either 1, 10, or 100 μg/kg of body wt per hour following an initial bolus of 1, 10, or 100 μg/kg of body wt respectively. Because PD results were similar for each dose of aldosterone, data have been pooled for analysis. As for group 2, measurements were commenced at least 2 hours after the bolus of aldosterone.

Microperfusion studies

Group 4: Aldosterone-infused intact animals (6 rats). Animals of this group were maintained on regular rat chow and tap water. Distal PD measurements were made under free-flow conditions and during distal microperfusion with artificial plasma ultrafiltrate (Table 1) through an initial control period and subsequently an experimental period during which aldosterone was administered i.v. in a dose of 10 μg/kg of body wt per hour after an initial bolus of 10 μg/kg of body wt. In these experiments, a single pipette was used for both microperfusion and PD measurement according to the technique described below.

Table 1. Composition of solutions

	Plasma ultrafiltrate	Artificial early distal fluid	Artificial late distal fluid
Sodium, *mmoles/liter*	148	48	35
Potassium, *mmoles/liter*	5	2	15
Calcium, *mmoles/liter*	1	1	1
Magnesium, *mmoles/liter*	1	1	2
Chloride, *mmoles/liter*	112	34	32
Bicarbonate, *mmoles/liter*	25	10	10
Mono hydrogen phosphate, *mmoles/liter*	4	3	11
Acetate, *mmoles/liter*	10	2	—
Sulphate, *mmoles/liter*	1	1	2
Mannitol, *mmoles/liter*	12	100	110
Urea, *mmoles/liter*	—	—	100
Osmolality, *mOsm/kg*	300	200	300
pH	7.4	7.4	6.0[a]

[a] pH was reduced to 6.0 by titrimetric addition of hydrochloric acid.

Group 5: Aldosterone-infused adrenalectomized animals (6 rats). This group received 0.9% sodium chloride drinking solution and were studied in an identical way to the animals of group 4.

Distal transepithelial PD measurements

Electrical measuring system. Sharpened glass micropipettes with tips of 3 to 5 μ in O.D. and filled with 3 M potassium chloride colored with 0.4% lissamine green (Roboz, Washington, D.C.) were used as puncturing electrodes. These electrodes were inserted into a Perspex chamber (also filled with 3 M potassium chloride), and electrical contact was established with a silver-silver chloride electrode mounted in the chamber and connected to the input of an electrometer (model 602, Keithley Instruments, Cleveland, Ohio). The electrometer was coupled to twin channel recorder (model B261, Rikadenki Kogyo Co. Ltd., Tokyo, Japan). To permit the injection of potassium chloride through the tip of the electrode, we equipped the Perspex chamber with a side-arm connected via a length of polyethylene tubing to a Gilmont micrometer syringe (Gilmont Instruments, Great Neck, New York) filled with light-weight mineral oil. A reference calomel electrode with a 3 M potassium chloride bridge made appropriate contact with the rat's tail via a small container of Ringer's bicarbonate solution. The reference side of the circuit included (*1*) a potentiometer, which was used to set a suitable zero for the electrical system, and (*2*) a voltage source, by which a fixed 10-mV potential could be applied to allow calibration of the electrical system. The methods for identifying distal tubular segments, performing PD measurements, and localizing electrode tips in a true intraluminal position have been described in detail previously [20, 24].

Microperfusion technique

Electrical shunting was minimized by using a single pipette (tip, 4 to 6 μ) for both microperfusion and PD measurement. The pipette, filled with artificial plasma ultrafiltrate, was held in a Hampel microperfusion apparatus, the barrel of which contained 3 M potassium chloride. A silver-silver chloride electrode was inserted into the barrel containing 3 M potassium chloride via a specially designed leak-proof side arm. This electrode was, in turn, connected to the input of an electrometer (Keithley

model 602). Microelectrodes were tested in vitro for streaming potentials arising from the microperfusion itself and were discarded if microperfusion at 55 nl/min resulted in a PD change of greater than 0.5 mV.

To exclude significant contamination of the perfusate by potassium chloride diffusing from the barrel of the microperfusion pump to the tip of the pipette, we collected serial samples of perfusate in vitro at regular time intervals over a 180-min period, and then we tested them for chloride concentration using the microcoulometric technique of Ramsay, Brown, and Croghan [36]. Because no progressive increase in perfusate chloride concentration with time was found, it was assumed that no significant contamination of the perfusing solution with potassium chloride was occurring over a 3-hour period. In any event, no single pipette was used in the in vivo experiments for a period exceeding 60 min.

Distal segments were identified by injecting a bolus of colored Ringer's bicarbonate into a randomly chosen proximal tubule. A proximal segment of the same nephron was punctured by a second pipette (tip, 8 to 10 μ 0.D.) containing Sudan-Black stained castor oil. Subsequently, the microperfusion electrode was positioned in the identified distal segment and the free-flow PD recorded. Following this measurement distal microperfusion was commenced at 55 nl/min. A long oil block was introduced to prevent proximal to distal flow and the proximal tubular fluid collected subsequently into the oil-filled pipette. The distal PD was recorded again under these conditions of distal microperfusion.

Statistical analysis. Standard variance analysis was used to determine differences between control and experimental periods and between groups. The significance of differences between a mean PD and zero was determined with Student's *t* test [37]. All results are expressed as the means ± SEM.

Results

Free-flow studies

Group 1: Distal nephron morphology group. In this group of animals, early distal segments had a mean PD of +8.9 ± 1.0 mV (N = 5), whereas late distal segments had a mean PD of −21.0 ± 2.0 mV (N = 19). Following PD measurements, 29 tubules were microdissected and submitted for electron microscopic examination to identify the type of epithelium present at the site of PD measurement. In 20 instances, the puncture site was clearly identified and the epithelial cell type determined. All 6 segments that had epithelium of distal tubular type had yielded a positive transepithelial PD and in 4 instances had been identified in vivo as early distal tubules. The remaining 14 segments possessed epithelium characteristics of cortical collecting duct, and in all instances these segments had yielded a large negative transepithelial PD. Of these 14 segments, 13 had been identified in vivo as late distal tubules. These data obtained in Ginger hooded rats are similar to those recorded previously for mutant Wistar rats [24] and indicate a similar functional and morphologic profile of superficial distal tubules in these two strains.

Group 2: Aldosterone-infused intact animals. In this group, during the control period, the mean early segment PD was +6.4 ± 1.1 mV (N = 31), and the mean late segment PD was −18.0 ± 1.5 mV (N = 26). With the administration of aldosterone during the experimental period, the early segment PD decreased slightly to +5.4 ± 0.8 mV (N = 23) (NS) and the late segment PD increased to −29.0 ± 1.9 mV (N = 26). a change which was highly significant ($P < 0.001$). (Table 2, Fig. 1).

Group 3: Aldosterone-infused adrenalectomized animals. During the control period, the mean early segment PD was +12.2 ± 1.1 mV (N = 49), a significantly higher positive value than that found in intact animals of group 2 ($P < 0.001$). Following administration of aldosterone, the early segment PD fell significantly to +8.0 ± 0.9 mV (N = 41) ($P < 0.001$). In late distal segments, the mean PD increased significantly from a control value of −0.7 ± 0.8 mV (N = 46) to −8.4 ± 1.0 mV (N = 41) with administration of aldosterone ($P < 0.001$) (Table 2, Fig. 1).

Microperfusion studies

Group 4: Aldosterone-infused intact animals (6 rats). In these studies, the microperfusion electrode was filled with artificial plasma ultrafiltrate, and all free-flow values were corrected for a liquid junction potential measured in vitro between artificial plasma ultrafiltrate and artificial early and late distal fluids of the compositions indicated in Table 1. The mean liquid junction potentials for early and late distal fluids were +3.5 and +4.4 mV, respectively. With these corrections, the control free-flow PD was +4.4 ± 1.0 mV (N = 20) in early distal segments and −18.8 ± 2.7 mV (N = 12) in late segments. Perfusion with artificial plasma ultrafiltrate yielded an early segment PD during the control period of −4.7 ± 0.4 mV (N = 24) and a late

Table 2. Free-flow transepithelial PD values in intact and adrenalectomized animals during control and experimental periods[a]

Group	Early distal segment PD, *mV*			Late distal segment PD, *mV*		
	Control (C)	Aldosterone (A)	*P* (C vs. A)	Control (C)	Aldosterone (A)	*P* (C vs. A)
2 (Intact)	+6.4 ± 1.1 (*N* = 31)	+5.4 ± 0.8 (*N* = 23)	NS	−18.0 ± 1.5 (*N* = 26)	−29.0 ± 1.9 (*N* = 26)	<0.001
3 (Adx)	+12.2 ± 1.1 (*N* = 49)	+8.0 ± 0.9 (*N* = 41)	<0.001	−0.7 ± 0.8 (*N* = 46)	−8.4 ± 1.0 (*N* = 41)	<0.001
4 (Intact)	+4.4 ± 1.0 (*N* = 20)	+4.3 ± 0.8 (*N* = 12)	NS	−18.8 ± 2.7 (*N* = 12)	−30.7 ± 2.2 (*N* = 20)	<0.005
5 (Adx)	+9.3 ± 1.1 (*N* = 22)	+5.8 ± 1.2 (*N* = 17)	<0.05	−7.0 ± 1.5 (*N* = 18)	−14.7 ± 1.7 (*N* = 18)	<0.005

[a] In each free-flow experiment of groups 2 and 3, 3 to 12 PD measurements were made per segment per period. Data for individual experiments are available from us.

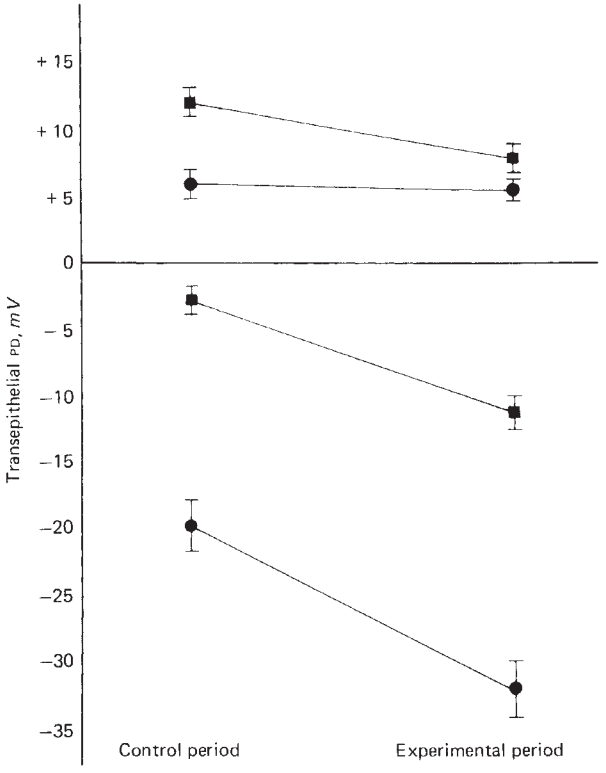

Fig. 1. *Summary of distal free-flow PD values (mean ± SEM) in control and experimental (aldosterone) period of groups 2 and 3.* Early distal PD's are shown in the upper section and late distal PD's in the lower section of the figure. *Closed circles* (●——●) denote group 2 (intact). *Closed squares* (■——■) denote group 3 (Adx).

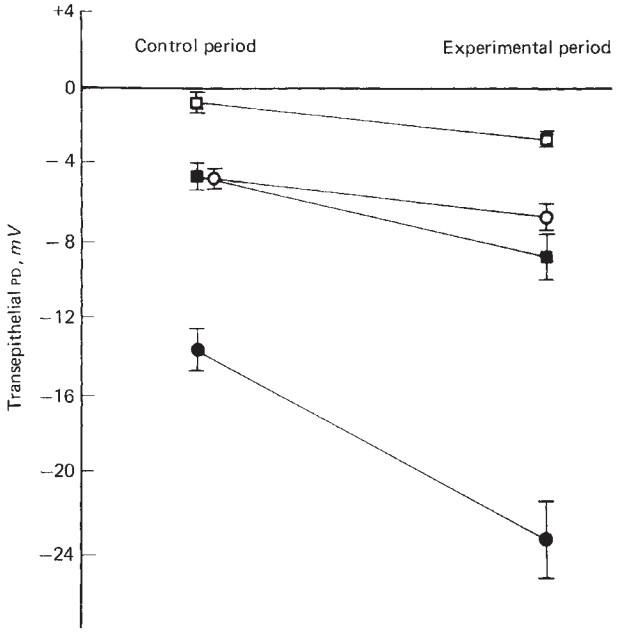

Fig. 2. *Summary of microperfusion PD values (mean ± SEM) obtained using the single pipette technique in control and experimental periods of animals of groups 4 and 5. Open circles* (○——○) denote group 4 (intact) early segments *closed circles* (●——●) denote group 4 late segments, *open squares* (□——□) denote group 5 (Adx) early segments and *closed squares* (■——■) denote group 5 late segments. Artificial plasma ultrafiltrate was used as the perfusate.

segment PD of −13.6 ± 1.1 mV (*N* = 16). After aldosterone, microperfusion with artificial plasma ultrafiltrate produced an early segment PD of −6.7 ± 0.7 mV (*N* = 24), whereas the late segment PD was −23.4 ± 2.0 mV (*N* = 19), this latter value being significantly higher than the control value (*P* < 0.001) (Fig. 2 and Table 3).

Group 5: Aldosterone-infused adrenalectomized animals (6 rats). In these animals, the control free-flow PD was +9.3 ± 1.1 mV (*N* = 22) in early distal segments and −7.0 ± 1.5 mV (*N* = 18) in late segments. Perfusion with artificial plasma ultrafiltrate yielded an early segment PD during the control period of −0.7 ± 0.5 mV (*N* = 27) and a late segment PD of −4.6 ± 0.7 mV (*N* = 25). After aldosterone, microperfusion with artificial plasma ultrafiltrate produced an early segment PD of −2.7 ± 0.3 mV (*N* = 28), whereas the late segment PD was −8.7 ± 1.1 mV (*N* = 25), both values being significantly higher than the control values (*P* < 0.005), but significantly lower than the corresponding values found in intact animals (*P* < 0.001) (Fig. 2 and Table 3).

Serum electrolytes. In the intact animals of groups 2 and 4, the mean serum sodium concentra-

Table 3. Free-flow and microperfusion transepithelial PD values in intact and adrenalectomized animals of groups 4 and 5 during control and experimental periods[a]

Group	Condition	Early distal segment PD, *mV*			Late distal segment PD, *mV*		
		Control (C)	Aldosterone (A)	*P* (C vs. A)	Control (C)	Aldosterone (A)	*P* (C vs. A)
4 (Intact)	Free-flow	+4.4 ± 1.0 (*N* = 20)	+4.3 ± 0.8 (*N* = 12)	NS	−18.8 ± 2.7 (*N* = 12)	−30.7 ± 2.2 (*N* = 20)	< 0.005
	microperfusion[b]	−4.7 ± 0.4 (*N* = 24)	−6.7 ± 0.7 (*N* = 24)	< 0.05	−13.6 ± 1.1 (*N* = 16)	−23.4 ± 2.0 (*N* = 19)	< 0.001
5 (Adx)	Free-flow	+9.3 ± 1.1 (*N* = 22)	+5.8 ± 1.2 (*N* = 17)	< 0.05	−7.0 ± 1.5 (*N* = 18)	−14.7 ± 1.7 (*N* = 18)	< 0.005
	microperfusion	−0.7 ± 0.5 (*N* = 27)	−2.7 ± 0.3 (*N* = 28)	< 0.005	−4.6 ± 0.7 (*N* = 25)	−8.7 ± 1.1 (*N* = 25)	<0.005

[a]In each microperfusion experiment of groups 4 and 5, two to seven PD measurements were made per segment per period per perfusion technique. Data for individual experiments are available from us.
[b]Microperfusion with plasma ultrafiltrate

tion was 146.6 ± 0.8 mmoles/liter and the mean serum potassium was 4.8 ± 0.1 mmoles/liter (N = 9), whereas in the adrenalectomized animals of groups 3 and 5, the mean serum sodium was 134.4 ± 1.0 mmoles/liter, and the mean serum potassium was 5.7 ± 0.1 mmoles/liter (N = 11). The difference in both sodium and potassium concentration between intact and adrenalectomized animals was statistically highly significant ($P < 0.001$ for both).

Discussion

Although a pronounced effect of mineralocorticoids on sodium reabsorption along the distal nephron has been demonstrated in earlier free-flow micropuncture studies [4, 9, 10], no associated variation in transepithelial PD has been observed. In 1965, Hierholzer et al [4] reported a highly significant decrease in sodium reabsorption along the distal tubule of adrenalectomized rats compared with control animals but were unable to detect any difference in the distal transepithelial PD between the two groups. Control animals had a mean distal PD of −45.2 mV, whereas adrenalectomized animals had a mean PD of −47.4 mV. These results contrast with those reported for other mineralocorticoid-sensitive epithelia such as the frog skin [14, 15], toad bladder [16–18], and mammalian colon [8, 38, 39], where an aldosterone-induced increase in transepithelial sodium movement is invariably accompanied by a significant increase in the transepithelial PD.

The failure to demonstrate an effect of aldosterone on the PD of the distal tubule in earlier in-vivo studies might readily be explained by recent data that suggest that the Ling Gerard electrodes, used traditionally for PD measurements in micropuncture work, frequently provide inaccurate readings because of inherent problems of high tip resistance, high and variable tip potentials, and difficulties with adequate tip localization [20, 23, 24]. In a recent study in which the electrophysiology of the distal tubule of the rat kidney was reexamined, Barratt et al [24] circumvented these problems by using electrodes with relatively large tips of 3 to 5μ in external diameter. These workers found that early segments of distal tubule had a significantly positive PD of approximately +4 mV, whereas late distal segments had a significantly negative PD of approximately −20 mV. Furthermore, subsequent electron microscopic examination of the punctured distal segments revealed that early segments with a positive PD possessed epithelium characteristics of true distal convoluted tubule, whereas late segments with a negative PD uniformly possessed epithelium characteristics of cortical collecting tubule [24]. Thus, in the rat, the distal tubule, defined as the nephron segment extending from the macula densa to the first branching with another tubule, appears to consist of at least two morphologically and functionally distinct segments. Similar morphologic variation has been reported by Woodhall and Tisher [35] for the distal tubule of several strains of rat. The finding by Barratt et al [24] of a positive PD in early distal segments and a PD of approximately −20 mV in late distal segments contrasts with the results of earlier micropuncture studies in which a negative PD was documented along the entire length of the distal convoluted tubule [40–48]. The discrepancy between these results can be attributed to differences in the electrodes used because in the earlier studies, PD measurements were made with Ling Gerard electrodes (tips < 1 μ O.D.), whereas Barratt et al used electrodes with tips of 3 to 5 μ. Controversy still exists as to the validity of PD

measurements made with these large tip electrodes [30–32]. But, we have recently reported data supporting the use of such electrodes for distal PD measurements [33]. Furthermore, large tip electrodes have now been used by several groups of investigators in studies of the electrophysiologic characteristics of a number of different nephron segments including the proximal tubule [20, 25], thin ascending limb of Henle's loop [26, 27], and medullary collecting duct [28, 29].

In the present study, we have reexamined the influence of aldosterone on the transepithelial PD of the distal tubule of the rat kidney using large tip electrodes. In addition, now recognizing the morphologic and functional heterogeneity of the distal tubule of the rat kidney, we have studied early and late distal tubules as separate segments.

Group 1 experiments were performed to ascertain that distal tubules of the Ginger hooded strain of rats used in the present study had similar electrophysiologic and morphologic characteristics to those previously described for the mutant Wistar rat [24]. Apart from a higher mean positive PD in early distal segments of Ginger hooded rats (+8.9 mV) compared with similar segments in the mutant Wistar strain (+3.7 mV), the potential profile of the distal tubule was similar in both strains. Similarly, electron microscopic examination of punctured segments revealed the same variation in morphology along the distal tubule of both strains. Early distal segments with a positive PD had epithelium characteristics of distal convoluted tubule, whereas late segments with a negative PD had epithelium characteristic of cortical collecting tubule.

Results obtained in subsequent groups demonstrate that aldosterone does exert a significant influence on the free-flow PD of superficial distal tubules in the rat kidney. Major PD changes were observed in late distal (cortical collecting) tubules. Intact animals of groups 1, 2, and 4 had a mean late distal PD of −19.2 mV, whereas aldosterone-deficient adrenalectomized animals of groups 3 and 5 had very low late distal PD's of −0.7 and −7.0 mV, respectively. When aldosterone was infused into both intact and adrenalectomized animals, a highly significant increase in late distal PD occurred. In group 2 intact animals, the PD increased from −18.0 to −29.0 mV, whereas in group 3 adrenalectomized animals, the PD increased from −0.7 to −8.4 mV (Table 2).

These mineralocorticoid-dependent PD variations were further investigated by performing a series of microperfusion studies (groups 4 and 5) designed to eliminate variations in distal fluid and ion delivery, which might possibly have influenced the free-flow PD results. Artificial plasma ultrafiltrate was used as the perfusate to exclude osmotic and ionic concentration gradients across the tubular wall. Any PD arising with this perfusate may thus be attributed to active ion transport. The much reduced negative PD in late distal segments of adrenalectomized intact animals (Fig. 2) and the significant increase in late distal PD with administration of aldosterone in both intact and adrenalectomized animals (Fig. 2) indicate that mineralocorticoids exert a direct effect on ion movement in the late distal tubule (cortical collecting tubule). Thus, the free-flow PD results cannot be explained solely by variations in distal solute delivery between different groups and/or experimental periods.

All of these data agree closely with those recently reported by Gross and Kokko for the in vitro perfused isolated rabbit cortical collecting tubule [19]. These workers found that perfusion with a solution resembling plasma ultrafiltrate yielded a mean PD of +1.6 mV in collecting tubules taken from adrenalectomized animals, whereas tubules taken from intact animals preloaded with deoxycorticosterone acetate (DOCA) generated a PD of −33.6 mV. The PD in isolated collecting tubules from rabbits ingesting a relatively high sodium diet was also close to zero, consistent with chronic suppression of aldosterone secretion. When aldosterone was added to the solution perfusing these same tubules, a slow but consistent increase in the transepithelial PD occurred, with a mean value of −19 mV being achieved after 84 min.

In the present study, the direction of PD change in response to aldosterone in collecting tubules is consistent with enhancement of active outward sodium transport, and although this PD response cannot be equated unequivocally with sodium movement, such as interpretation is well founded experimentally [14–18, 49–53].

Although our data indicate major aldosterone-related variations in PD in late distal or cortical collecting tubules, it should be recognized that smaller but nevertheless significant changes in PD also occurred in early distal segments in response to alterations in mineralocorticoid status. Early distal segments of the adrenalectomized animals of groups 3 and 5 had a significantly higher free-flow mean positive PD than did similar segments in the intact animals of groups 2 and 4 ($P < 0.001$) (Fig. 1). Furthermore, acute administration of aldosterone to adrenalectomized animals in groups 3 and 5

resulted in a significant reduction in the magnitude of the free-flow positive PD of early distal tubules. We have shown previously that the positive PD in early distal segments is generated by inward passive sodium and potassium diffusion but is attenuated to some degree by an amiloride-inhibitable outward active sodium movement [33, 54]. The present results suggest that the active sodium transfer mechanism is also mineralocorticoid-sensitive. Although it is possible that the small free-flow PD variations observed in early distal segments in response to changes in mineralocorticoid status might reflect alterations in the ionic composition and/or volume of tubular fluid delivered to the distal tubule, the results of the microperfusion studies of groups 4 and 5 indicate that aldosterone does exert a direct effect on early distal transport (Fig. 2).

Finally, our observations indicate that early distal segments of the rat kidney are functionally rather different than are distal convoluted tubules of the rabbit kidney. Although we find that in vivo, under free-flow conditions, the rat early distal tubule has a significantly positive PD that appears to be influenced to some extent by aldosterone, Gross et al [19, 50] have reported that the rabbit distal convoluted tubule, when perfused in vitro with a solution resembling plasma ultrafiltrate, generates a large negative PD of the order of -35 mV that is totally independent of any effect of aldosterone. These data clearly imply caution in extrapolating results from one species to another.

Acknowledgments

This work was supported by grants from the National Health and Medical Research Council of Australia, The Queen Elizabeth Hospital Research Foundation, the Australian Kidney Foundation, and the Flinders University. Ms. S. Liddicoat, G. Hearne, and D. Turner helped prepare the manuscript.

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